AN ILLINOIS CASE OF FILARIASIS

BY

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THESIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY

SUPERVISION BY BERTHA LANGWILL

ENTITLED AN ILLINOIS CASE OF FILARIASIS

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR

THE DEGREE OF MASTER OF SCIENCE

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In Charge of Thesis

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Recommendation concurred in*

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on
Final Examination*

*Required for doctor's degree but not for master's

OUTLINE.

INTRODUCTION

At the suggestion of Professor Ward, under whose direction this paper was prepared, the writer visited Dr. J. S. Mason of Urbana and became aquainted with a case of filariasis in a woman, a trained nurse, who was a native of Georgetown, British Guiana.

On the evening of October 17, 1917 at 9:40 P.M., with Dr. Mason, the nurse was visited and six blood smears made. On subsequent occasions - November 17, at 7:30 P.M. and January 24, at 8:30 P.M. - further smears were made by myself, also drops of blood were obtained and ringed with vaseline for the examination of living forms; then later on April 10, at mid-day, eight thick smears were made from the blood of the nurse by Dr. Graves of Sidney, Illinois, while she was attending one of his patients.

The history of the case as obtained is as follows:

Miss B. was a native of Georgetown, British Guiana; there she obtained her nurse's training in the government hospital. While in South America she experienced repeated febrile attacks, attributed by her to malaria, but which more probably were filarial attacks. At no time has she had filarial abscesses, vomiting, blood or chyle in the urine. After leaving British Guiana in 1913 she went to Canada, and there spent three years in nursing at Montreal. From there she came to Urbana, and in September or October (she could not remember which) of 1916, she experienced her first known attack of filariasis. It was sudden. Acute liver, peritoneal, and abdominal pains occurred accompanied by vomiting; there were chills and fevers, pain in the back - not in any exact location, but a general backache - hard swellings appeared in both groins, urine was colored but not with blood. The case was diagnosed as strangulated hernia, and an operation advised; but the correct di-

agnosis of filariasis was determined by Dr. Graves, himself a native of British Guiana. Having practiced medicine there he was thus well acquainted with the symptoms and the disease; the proof was quickly given by a microscopic examination of the blood.

The treatment used was complete rest, and the groin swellings were painted with guaiacol. She was confined to bed for three weeks. The second attack occurred in January 1917, the third in March 1917. These came suddenly, entirely unexpectedly, and with great severity. The symptoms were identical with those of the first, but the duration of each was but a week. At the time of this writing no further seizures have occurred since the date of March 1917.

In the smears made, filaria embryos were most numerous, with the exception of those made at noon on April 10, at which time but two parasites were found on the eight thickly smeared slides. This is indicative of a pronounced periodicity. All the smears were examined first before staining, and then subjected to the various methods of technic explained further in Part VI.

GEOGRAPHICAL DISTRIBUTION OF FILARIASIS WITH REFERENCE TO SOUTH AMERICA, AND ESPECIALLY BRITISH GUIANA.

Filaria bancrofti. The parasite is associated with a very large number of patholgical conditions, all of which are characterised by more or less severe affection of the lymphatic system. Of these various diseases, due more or less directly to the Filaria nocturna, Manson has enumerated the following:- ulcers; lymphangitis; varicose groin glands; varicose axillary glands; lymph scrotum; cutaneous and deep lymphatic varix; orchitis; chyluria; elephantiasis of the leg, scrotum, vulva, arm, mamma, and elsewhere; chylous dropsy of the tunica vaginalis; chylous ascites; chylous diarrhoea, and probable other forms of disease depending on obstruction or varicosity of the lymphatics, or on death of the parent filariae.

Of all these varied affections, elephantiasis is by far the most widely spread. In South America elephantiasis is said to be endemic on the coast and marshy plains of Guiana, in some parts of Brazil, and on the shores of Colombia, Venezuela, and Peru. In British Guiana filaria affections appear to be particularly common. Danials found the Filaria nocturna in 52 out of 348 persons examined for it at Georgetown, and Ozzard found it in 28 out of 100 persons in whom it was looked for at New Amsterdam. It is also of comparatively frequent occurrence in some parts of Brazil; one observer has found it at Bahia in about one-twelfth of the persons examined for it.

There are a number of necessary factors associated with the determining of the geographical distribution of diseases associated with the Filaria nocturna. There is the specific filaria itself; the specific mosquito (or perhaps several species of mos-

quito) in which it undergoes its extra-human phase; there must be a susceptible population, and a set of physical conditions favorable both to the life of the parasite, to the life of its insect host, and to its easy passage from the latter to the human subject and again from the human subject to the mosquito.

A close examination of Georgetown, British Guiana, with reference to the breeding places of the myriads of mosquitos always present, was reported by Wise (1911). "It seems almost impossible to realise that during the wet season over 70 per cent. of all premises in this city are breeding countless myriads of these pests, and that during the dry season nearly 60 per cent. are equally incriminated. This state of affairs, occurring as it does in a city priding itself on being up to date, is scarcely to be credited, and reveals the urgent necessity for vigorous and prompt action by those responsible for the health of the city. 2560 premices were visited; 1490 of those were breeding mosquitos, Stegamyia fasciata was found breeding in 1362 instances; Culex fatigans 250, Anapheles 21, and other mosquitos 25."

It was in 1879 that Sir Patrick Manson, working in China, made the discovery that the Filaria is extracted from the blood of a filarial patient by the mosquito, and that in the body of the mosquito the parasite undergoes another cycle of life. Low in 1900 demonstrated that the young embryos get into the proboscis of the mosquito, and in this way, when the mosquito bites, the embryos are injected into a healthy person, thus infecting him. The mosquito infected with Filaria spreads filariasis in a similar way to that in which the mosquito infected with the Malarial parasite spreads malaria.

Jackson (1907) reports that the particular variety of mos-

quito most concerned in filarial distribution is Culex fatigans, an extremely common variety in most tropical countries. It is possibly also distributed by one or more of the Anaphelina. This has been proved many times in yellow fever and malaria investigations as reported by Simpson (1917).

Filaria diurna. The embryo resemble those of Filaria nocturna in all their physical characters, but differs from it in that it is found in the blood, not during the night, but during the day. The adult is found in the connective tissues, and seems to wander about from one part of the body to another. It is most usual to find it under the conjunctiva, or in the connective tissue in the neighborhood of the eye, or just under the skin on the bridge of the nose. The life-history of this Filaria is unknown; nothing is known of the way in which it leaves the human host, or whether it passes part of its life-cycle in some insect host. This parasite is only found in the West Coast region of Africa where it is rather common.

Filaria Ozzardi. This nematode embryo has a sharply pointed tail, and no sheath. Braun (1906) records that the larvae of Filaria demarquayi are indistinguishable from those of Filaria ozzardi, and are only half the length of the larvae of Filaria bancrofti. It is found in the blood of certain aboriginal Carib Indians in British Guiana.

Filaria perstans. The larva of Filaria perstans observes no periodicity; it is much smaller than either Filaria diurna or nocturna. The absence of a sheath, an abruptly rounded caudal end, and the power of locomotion are physical characteristics which markedly distinguish it from the other Filariae. The pathological effects are uncertain. At one time Filaria perstans

was incriminated as a possible factor in the etiology of sleeping sickness, but recent researches have proved that it has no causal connection with the disease. This parasite appears to have a wide geographical distribution. In South America, Filaria perstans is very common amongst the aboriginal Indians in the interior of British Guiana. However, it is not found in Georgetown and in New Amsterdam.

NOMENCLATURE OF THE PARASITES

The term "Microfilaria" has been suggested by LeDantec to designate the larval forms which circulate in the blood. That term has been adopted as being a convenient expression.

Pathological significance of importance is connected only with the forms Filaria bancrofti and Filaria loa. Because of the periodicity associated in most cases with these two, the terms Filaria nocturna and Filaria diurna have been respectively applied to each. Manson has proposed that the larval forms be called Microfilaria bancrofti and Microfilaria loa, but many other investigators speak also of Microfilaria nocturna and Microfilaria diurna, so a number of terms are employed.

Synonymous terms:

Adult form:Filaria bancrofti or Filaria nocturna
Embryo form:Microfilaria bancrofti or Microfilaria nocturna
Adult form:Filaria loa or Filaria diurna
Embryo form:Microfilaria loa or Microfilaria diurna

As the larval forms of Filaria bancrofti and Filaria loa are morphologically very similar - both are found in the peripheral blood, are of very nearly like size, possess sheath, and superficial structure is almost identical - careful measurements and

morphological differential diagnosis are essential for the identification.

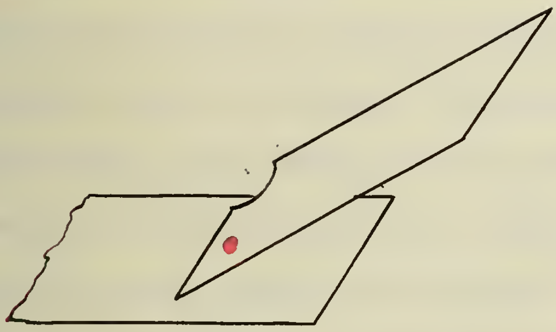
EXAMINATION OF LIVING MICROFILARIAE

On January 24 at 8:30 P.M. twenty-five blood smears were made; another slide on which a drop of blood was placed was not smeared, but ringed with vaseline, a cover-glass was put over it thus sealing it and preventing rapid drying. When the latter was examined the next morning under the microscope the parasites appeared as long, slender, graceful worms possessing a most remarkable degree of activity. They were wriggling furiously, lashing the corpuscles; others had moved out into the vaseline and could be most carefully watched as their movements were thus greatly impeded; but the largest number were found in the clear serum. They were of a pearly-gray color, almost transparent, with perhaps the faintest suggestion of a yelowish tone in certain lights. At first glance, the general impression of the animal was that of a thin, transparent tube, through which was constantly circulating a stream of liquid. The head (cephalic end) was gracefully rounded, while the tail (caudal end) gradually tapered, and ended in a fine pointed extremity. The worm was cylindrical in shape, of regular outline, and consisted of a central body enveloped in a distinct loosely fitting sheath, which - as DaCosta (1905) so aptly described it - was about as much too large for the body as the thumb of an adult's glove would be for the little finger of a child. That part of the sheath temporarily unoccupied by the body was found to collapse, was folded upon itself and trailed after the worm at either or both extremities as a twisted, whip-like ribbon. The greater part of the body appeared to be of homogeneous structure, but by the second day coarse granulations began

to appear like a stippling on its surface. A series of fine striations, like the milling on a coin appeared to run along both edges of the body at right angles to its long axis. A "viscus", appearing as an irregular mass of granular material, occupied the posterior part of the central third of the worm's body, running parallel to its long axis for some distance. Upon careful examination with an oil-immersion objective, rhythmical dimpling or puckering movements could generally be observed at the tip of the cephalic end of the embryo. This movement was probably the alternate covering and uncovering of the head end by the delicate six-lipped prepuce of which DaCosta (1905) makes reference. The sudden projection and equally rapid retraction of a filamentous fang or tongue-like organ from the worm's uncovered head was also noted in a few instances, but this characteristic was so difficult to make out that it was usually looked for in vain. The slide, which was kept at regular room temperature, was examined each day. After the fifth day many had lost their sheaths, more were found in the serum, while others could be readily seen by the constant agitation of the corpuscles with which they were covered. By February 4, or twelve days after the blood had been taken, three or four were still found to be alive, but their movements were very slow, and the corpuscles about them were scarsely stirred. On the following day, after a most careful examination of the slide, none remained which could be judged as living.

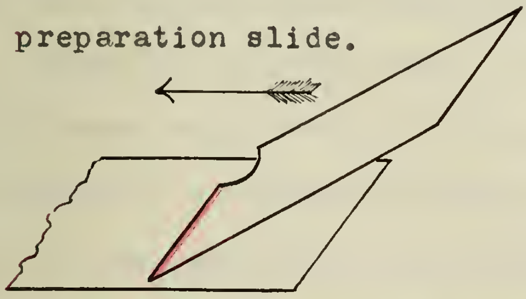
TECHNIC - DRIED BLOOD SMEARS.

The method followed for the making of smears was that laid down by Neuman and Mayer (1914). They were made best on slides, rather than cover-glasses, and the strip of glass which was used in spreading the blood film had a corner broken from it, so that the smear was kept from the edge of the slide. Another point which is not always observed in the rules of technic, is the direction in which the distributer is moved. Instead of being pushed forward so that the blood layer follows behind the edge, it is often drawn in the opposite direction, so the parasites, and blood corpuscles as well, become rolled and assume unnatural positions.

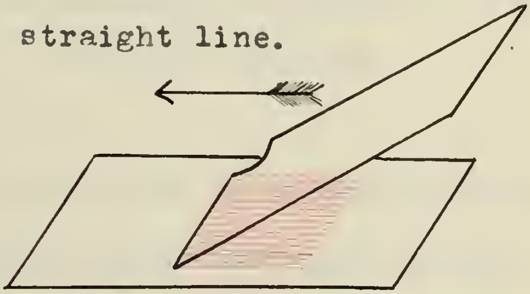


The distributer is set obliquely, a little in front of the drop of blood found on the preparation slide.

The distributer is pushed backward in the direction of the arrow until the drop all adheres in a straight line.



The distributer is pushed forward with equal pressure, and the adhering blood layer follows behind the sharp edge.



The spreading of the blood layer during the smear is shown.

The films were dried in air and could be kept indefinitely before stained. Different workers use their own methods of staining and it is according to whether one only wishes to find, by the stain, the presence and form of the parasite, cr if the more exact anatomy should be studied, or the differential diagnosis placed.

Like DaCosta (1905), I found the technic suggested by Manson (1917) and many others (washing out the erythrocytes with water, drying, fixing in alcohol, and then staining) to have proved unreliable in most cases, for the filaria embryos were washed out too although where that method did prove successful, the quality of the results were far superior to those obtained by direct fixing and then staining without the removal of the hemoglobin, but the numbers were so greatly diminished, it was not economical in cases where many were desired for measurement. A variety of stains and several combinations were tried: methylene blue and eosin, azurII, hemotoxylin, eosin, and giemsa - the latter in most cases gave the best results - and the method followed was that published by Bausch and Lomb, Optical Company, and slightly modified.

Giemsa's Stain for Malaria, "Old Formula".

1. Fix the air-dried preparation in absolute alcohol for 15 minutes, or in pure methyl alcohol for 2 or 3 minutes.
2. Remove from alcohol and dry in air.
3. Prepare a freshly diluted staining fluid by adding one drop of the stain to 1 cc. of distilled water and shake gently. This dilution should be made just before the staining is done.
4. Cover the smear with the solution just prepared and allow it to remain for from 10 to 15 minutes. Wash in distilled water.
5. Dry in air, and mount in balsam for examination.

MORPHOLOGY OF STAINED MICROFILARIA.

Not all the stains used brought out each morphological point with equal clearness, but after a careful study of over one hundred animals the main histological features of the embryo structure were determined.

The <u>cuticle</u> is transversely striated, best seen in the clear areas outside the central column of nuclei and at the nerve ring and pores.

The <u>subcuticular cells</u> (matrix cells of Rodenwaldt; muscle cells of Fülleborn) were spindle shaped, extending in the direction of the long axis of the body.

The <u>nerve ring</u> can best be described as a break in the nuclear column extending across the body at a distance of 19.7 % of the entire length of the animal from the head.

The <u>excretory pore</u>, 29.5 % of distance from head, is similar in appearance to the nerve ring, though not so clearly defined, but by some methods of staining the characteristic vacuole appears opening to the exterior. Lying just posterior to the pore is the <u>excretory cell</u>, slightly larger than the cells of the central column.

The <u>anal pore</u>, 82.8 % of length from head, did not in any case show as clearly even as did the excretory pore. Close to the pore, and directly anterior to it lie three small <u>genital cells</u> in serial form; another cell belonging to this group, the chief genital cell (g.1), is slightly larger than the others and lies about 71.1 % of distance from the head.

The <u>internal body</u>, "Innenkörper", viscus, or reserve material, extends between 40 % and 60 % of the length from the head, Only the giemsa staining showed this granular, irregular, strand-like

body.

The <u>central column of nuclei</u> are the main feature, extending the entire length of the body except for a short space immediately at the head end, and terminating in most cases at a point about 5 % of the entire length from the caudal end. A distinctive feature of all preparations show them separated by a clear space from the cuticula. The nuclei themselves are small and in normal cases are oval in shape; but in somewhat shrunken specimens they are crowded together forming a more compact column and so their individual characteristic appearance is lost.

MEASUREMENTS AND GRAPHS.

One hundred filaria embryos, which had been stained by various methods were drawn with the camera lucida. As the lengths were found to vary greatly, the oculars and objectives of the microscope were changed with the size of the animals, so that the entire filaria would always be found in the field, and so facilitate the drawing of it with the camera lucida.

Combinations of magnifications used:	Number of individuals:
Ocular 4, oil-immersion 12	15
Ocular 4, objective 8	68
Ocular 3, objective 8	17
	100

The animals were measured from the camera lucida drawings by the use of dividers and the scales obtained from a stage micrometer at the different magnifications used, as indicated above.

As the relative lengths of the anatomical fixed points in the different species are supposedly very useful for differential diagnosis, Fülleborn's (1913 b) scheme of measurement was observed and from these following points measurements were made:

1. The middle of the nerve ring (this corresponds to the "break in the continuity of the cells" of the English.)
2. The middle of the excretory pore (this corresponds to the "V-spot" of the English.)
3. The middle of the anal pore (genital pore or tail spot.)

In addition the commencement and ending of the stained inner-body (Innenkörper or central viscus) was noted.

The ratio of the <u>distance of each structure from the head end</u>, to that of the <u>entire length of the body</u> was found in percent

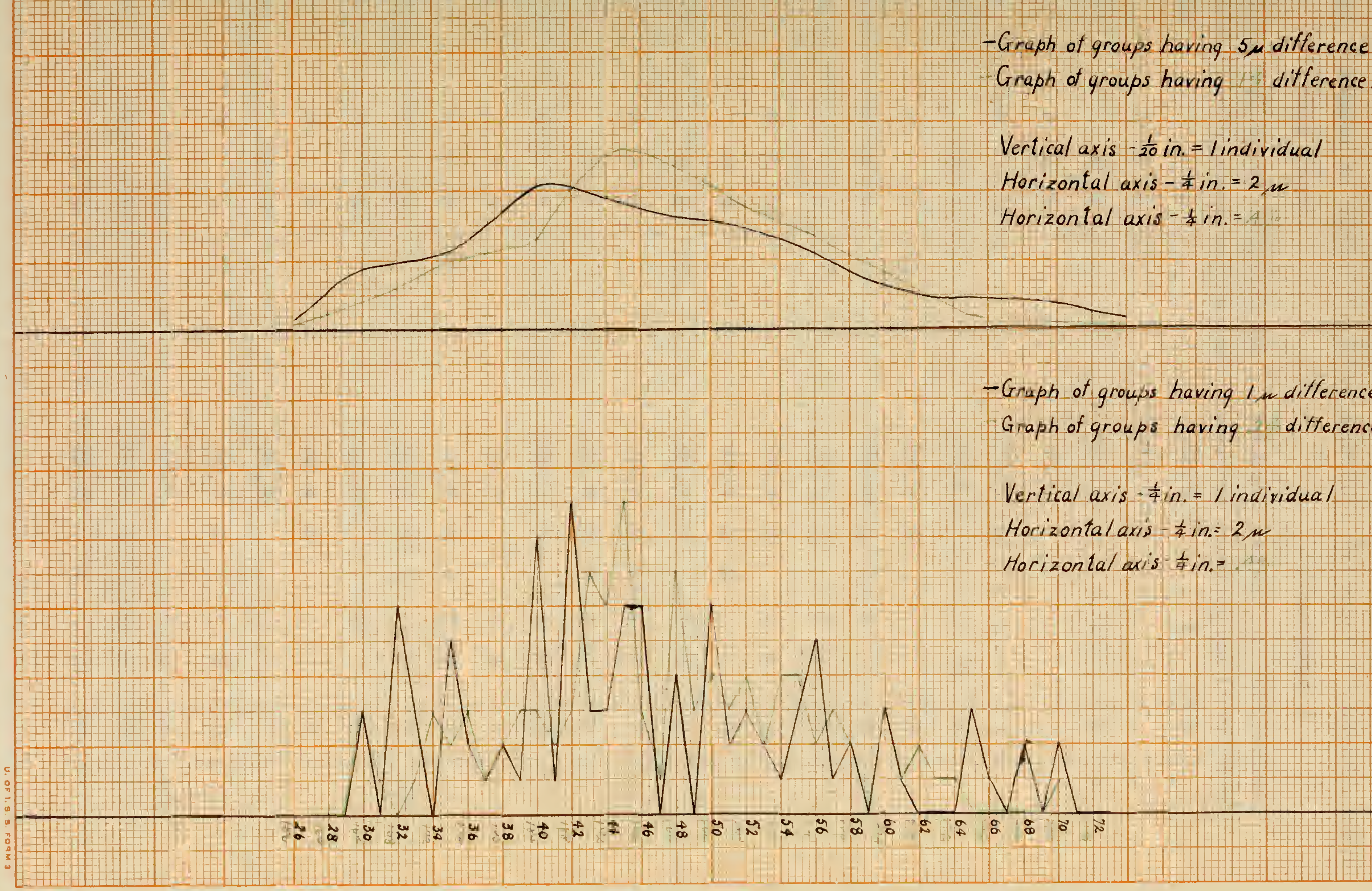
Actual distance of Nerve Ring from Head			Percentage distance of Nerve Ring from Head		
Distance	Number of Individuals		Percent	Number of Individuals	
30 µ	3	9	16.4 %	3	4
31			16.6	1	
32	6		16.8		
33	3	11	17.0	1	10
34			17.2	3	
35	5		17.4	2	
36	2		17.6	3	
37	1		17.8	1	
38	2	21	18.0	2	13
39	1		18.2	3	
40	8		18.4	3	
41	1		18.6	2	
42	9		18.8	3	
43	3	18	19.0	7	26
44	3		19.2	6	
45	6		19.4	9	
46	6		19.6	3	
47			19.8	1	
48	4	15	20.0	7	21
49			20.2	3	
50	6		20.4	4	
51	2		20.6	3	
52	3		20.8	4	
53	2		21.0	2	
54	1		21.2	4	

Actual distance of Nerve Ring from Head (cont.)			Percentage distance of Nerve Ring from Head (cont.)		
Distance	Number of Individuals		Percent	Number of Individuals	
55	3	12	21.4 %	4	15
56	5		21.6	2	
57	1		21.8	3	
58	2	6	22.0	2	8
59			22.2		
60	3		22.4	3	
61	1		22.6	1	
62			22.8	2	
63		4	23.0	1	2
64			23.2	1	
65	3		23.4		
66	1		23.6		
67			23.8		
68	2	4	24.0		1
69			24.2		
70	2		24.4	1	
	100			100	

A comparison of the actual and percentage distances of the Nerve Ring from the Head, shows that the data for the two when plotted follow each other quite closely, but both are multimodal curves. If the number of observations were increased, some of the modes would probably disappear and the curve would become more nearly normal. The minimum measurement for the actual distance of the Nerve Ring from the Head is 30 microns, maximum 70 microns, with a variance of 133 1/3 %, but the difference between

maximum and minimum of percentage distance is but 8 % (24.4 % and 16.4 %).

Graph of Actual and Percentage distance of Nerve Ring from Head.



Actual distance of Excretory Pore from Head

Distance	Number of Individuals			
44		1	1	10
46	3	1	4	
48		1	1	
50	3	1	4	
52	10		10	23
54		1	1	
56	2		2	
58	3	2	5	
60	5		5	
62	6	4	10	27
64		5	5	
66	4	4	8	
68	2	1	3	
70		1	1	
72	6	1	7	17
74		2	2	
76	1	1	2	
78	1		1	
80	5		5	
82	5		5	11
84		3	3	
86	1		1	
88				
88	2		2	
92		1	1	

Percent. distance of Excret. Pore from Head

Percent	Number of Individuals					
23.8 %			1		1	4
24.2						
24.6			1		1	
25.0						
25.4	1			1	2	16
25.8				2	2	
26.2	2				2	
26.6	1	1	1		3	
27.0	3		2	1	6	
27.4	1		1	1	3	
27.8	1	2			3	41
28.2	2		3	4	9	
28.6	2	1	2	2	7	
29.0	2	1	3	2	8	
29.4	3		5	6	14	
29.8			4	3	7	22
30.2	2	1	1	3	7	
30.6	3	1		1	5	
31.0			2		2	
31.4	1				1	
31.8						14
32.2	1			2	3	
32.6			2		2	
33.0	4	1	1	1	7	
32.4		1		1	2	

Actual distance of Excretory Pore from Head (cont.)

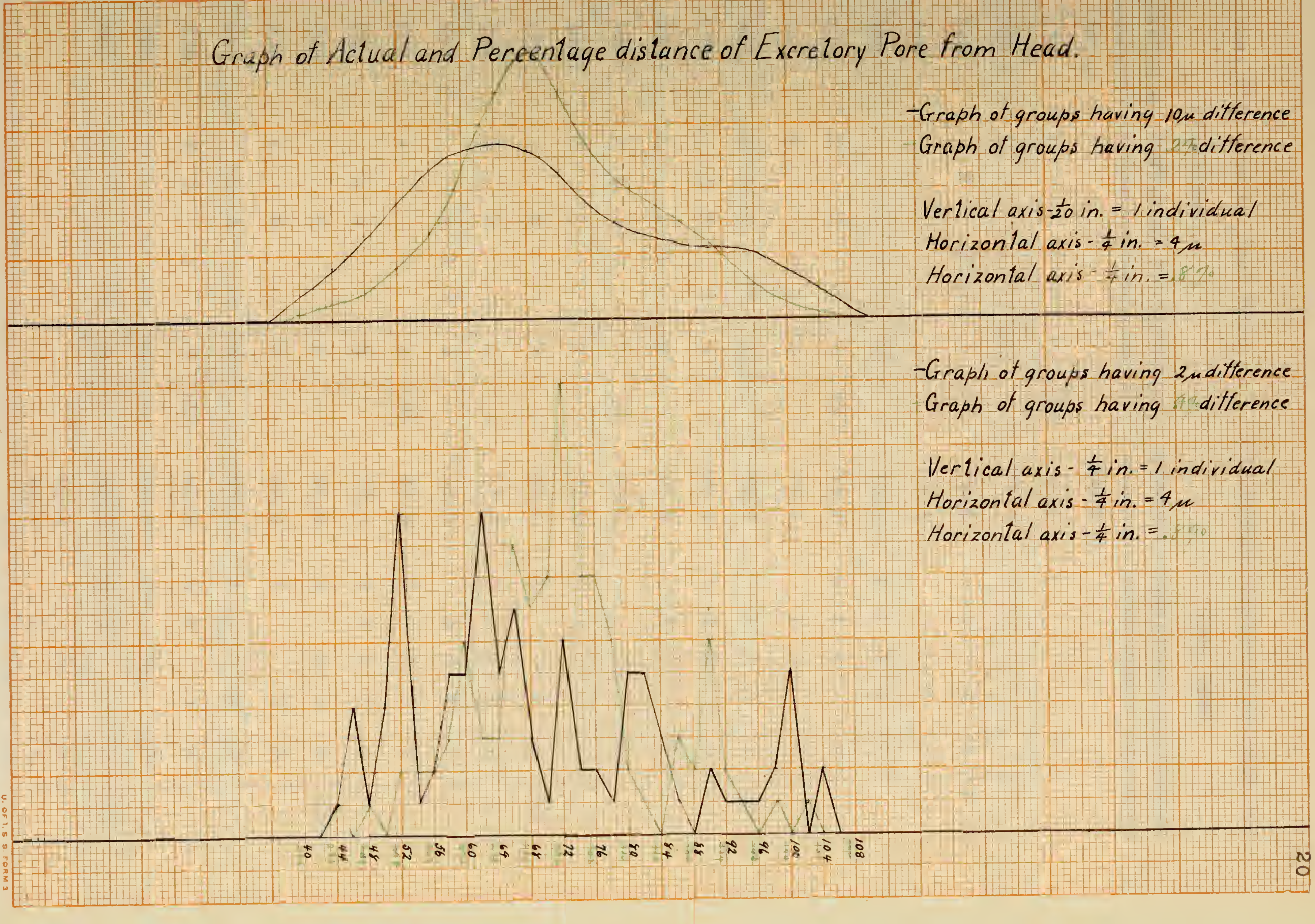
Distance	Number of Individuals			
94 μ		1	1	10
96	1		1	
98	2		2	
100	4	1	5	
102				2
104		2	2	
			100	

Percent. distance of Excret. Pore from Head (cont.)

Percent	Number of Individuals				
33.8 %		1		1	3
34.2					
34.6			1	1	
35.0					
35.5	1			1	
				100	

The maximum and minimum of the actual distance of the Excretory Pore from the Head, as found in this data, show a difference of 60 microns or 136 4/11 % (104 microns and 44 microns). The curve is very nearly normal with the exception of a second smaller mode near the end of the graph. The percentage curve is more uniform, but does not closely follow the actual distance curve. The variance here is 11.6 % between the maximum and minimum of the percentage differences.

Graph of Actual and Percentage distance of Excretory Pore from Head.



Actual distance of Anal Pore from Head

Distance	Number of Individuals						
128 µ							
133	1		1	1		4	12
138	1		1	1	1	4	
143		2		1	1	4	
148					1	1	
153			1	2	2	5	
158	2		2		1	5	25
163	1			1	1	3	
168	3	1	4		2	11	
173		3	2		1	6	
178	1		2	1		4	
183	1	2	5			8	25
188	4		1			5	
193			2			2	
198	2		3			5	
203			1	1	1	3	
208	1					1	13
213		1	1			2	
218			2			2	
223	1		2			3	
228		2	1		1	4	
233	2	1		1		4	15
238					1	2	
243	1				1	2	

Percentage distance of Anal Pore from Head

Percent	Number of Individuals					
76.8 %						
77.2	1				1	
77.6						
78.0		1		1	2	8
78.4			1		1	
78.8	1	1	1	1	4	
79.2	3	1	1		5	
79.6		1	1		2	
80.0		1	1	2	4	20
80.4	1	3	1		5	
80.8		1	3		4	
81.2			1		1	
81.6	1	1	3	2	7	
82.9	2			2	4	30
82.4	3	1	2	1	7	
82.8		5	3	3	11	
83.2	2		1	3	6	
83.6	2	2	1	3	8	
84.0	1		3	1	5	25
84.4	1			2	3	
84.8	1	1		1	3	
85.2	2	1		1	4	
85.6	1		2		3	
86.0	2	1			3	12
86.4			1		1	
86.8		1			1	

Actual distance of Anal Pore from Head

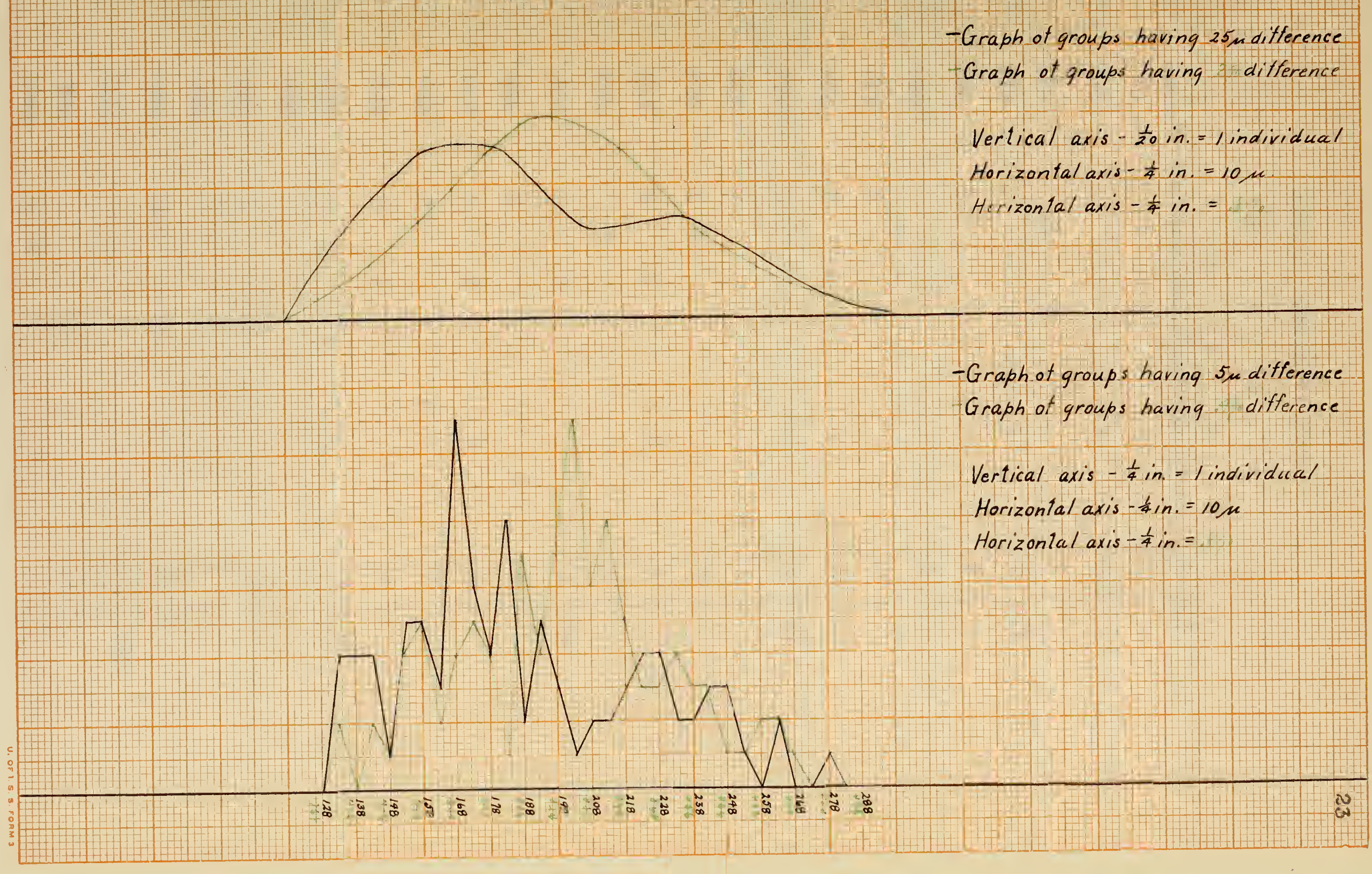
Distance	Number of Individuals				
248 ✓	3			3	
253	1	2		3	
258	1			1	9
263					
268		1	1	2	
273					
278					
283	1			1	1
				100	

Percentage distance of Anal Pore from Head

Percent	Number of Individuals					
87.2 %	1		1		2	
87.6		1		1	2	
88.0	1				1	5
					100	

A nearly normal curve is found in the plotting of the percentage distance of the Anal Pore from the Head. 10.8 % difference (88.0 and 77.2 %) was found between the maximum and minimum, with an average at 82.8 %. The data of the actual distances, when plotted, show clearly that with actual distances the variance is so great - here 112.7 % - that only the percentage distances are of value.

Graph of Actual and Percentage distance of Anal Pore from Head



Actual Length of Body

		1	6	2	7	3	8	4	9		
160 w	1	1		1						3	? 3
165	1						1		1	3	11
170		1		1		1				3	
175	1						1			2	
180	1			1						2	
185										1	
190	3			2						5	23
195	2		2				1		2	7	
200	3			1						4	
205	2								2	4	
210	1	2								3	
215	2		3				2			7	24
220		2		4						6	
225	1		2						1	4	
230	2			1				1		4	
235	1		1				1			3	
240	2			1		2				5	12
245	2		1							3	
250		1		1						2	
255											
260	1			1						2	
265											12
270	1			1				1		3	
275		1					1			2	
280				1		1				2	
285	1	2					2			5	

Actual Length of Body (cont.)

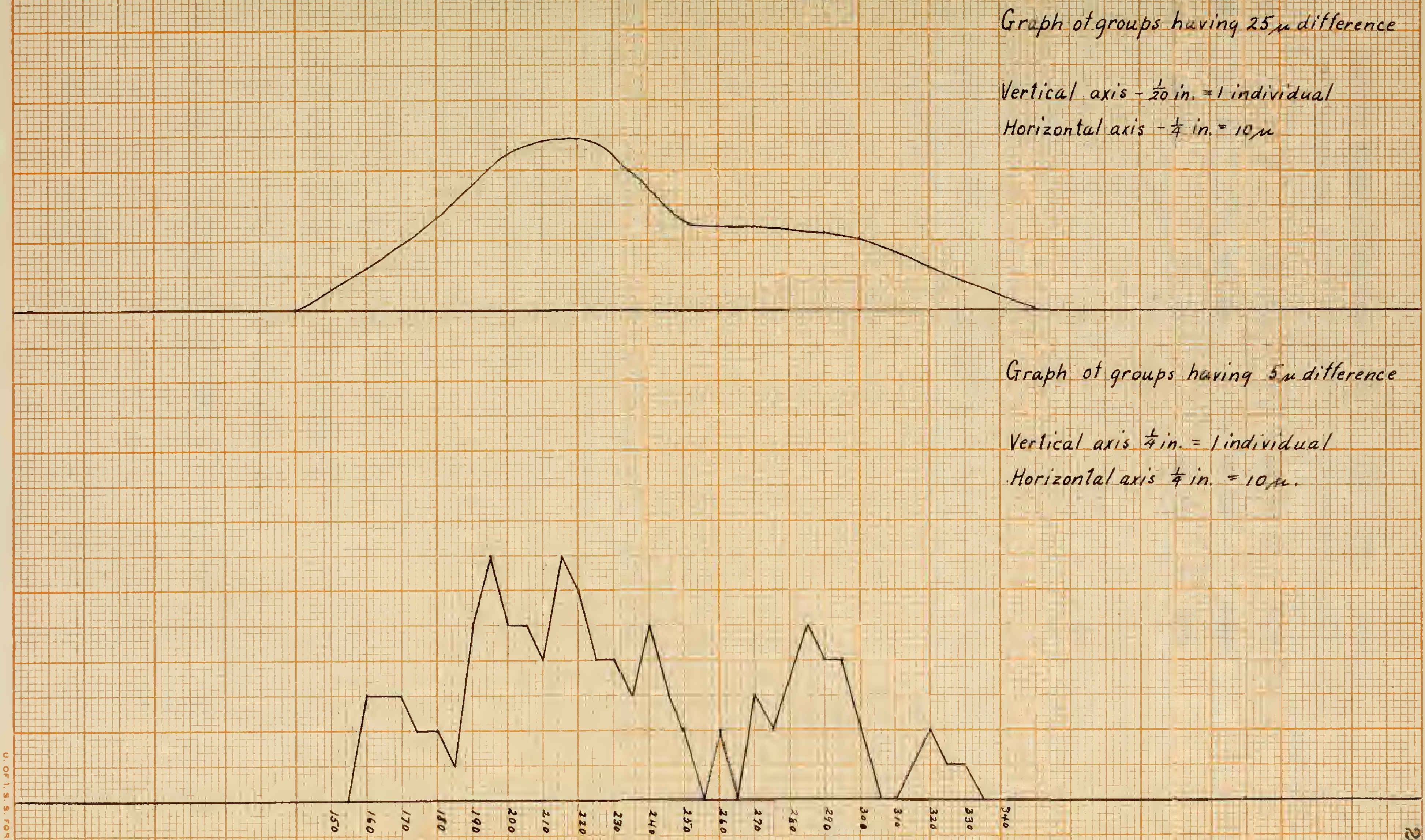
		1	6	2	7	3	8	4	9		
290 μ	2	1		1						4	10
295	2						1		1	4	
300		2								2	
305											
310											
315							1			1	5
320	1	1								2	
325							1			1	
330			1							1	
										100	

A study of the length of one hundred animals as measured in microns, shows a minimum of 160 microns, with a maximum of 330 microns, which gives a variance of over 110 %. When plotted, the curve was found to rise normally; the greatest number of individuals - being 32 - of nearly like measurements, were found between the lengths of 200 microns and 230 microns; the curve then begins to descend, but as 22 parasites are found between 270 and 300 microns, with but 15 between 230 and 270 microns, the graph does not fall rapidly, and the average is found to be at 232.88 microns.

These notations were tabulated for comparison with similar percentage measurements of Fülleborn (1913 b).

The maximum, minimum, and average of the measurements are as follows:

Graph of Actual Length of Body



	Nerve Ring	Excretory Pore	Anal Pore	Length
Average	19.75 %	29.50 %	82.82 %	232.88
Minimum	16.40	23.80	77.20	160
Maximum	24.40	25.40	88.00	330

COMPLETE TABLE OF MEASUREMENTS.

Abbreviations used: Len. - Length; Dis. - Distance from Head; % - Percentage of Distance from Head; N.R. - Nerve Ring; Ex.P - Excretory Pore; I.B. - Inner Body; A.P. - Anal Pore.

Len.	N.R.		Ex.P.		I.B.					A.P.	
	Dis.	%	Dis.	%	Dis.		%		Len.	Dis.	%
235 μ	50 μ	.212	75 μ	.319	130 μ	172 μ	.55	.73	42 μ	200 μ	.853
243	44	.190	66	.270	118	173	.48	.711	55	190	.783
222	50	.225	73	.328						181	.816
240	50	.208	78	.325	121	153	.504	.637	32	208	.866
211	48	.227	68	.322						168	.794
205	40	.195	59	.287	112	145	.546	.707	33	170	.829
230	48	.208	70	.304	123	154	.51	.64	31	198	.860
220	43	.195	66	.300	120	146	.53 m	.646	26	170	.772
227	45	.198	65	.286	123	161	.525	.688	38	185	.814
222	40	.180	62	.279	125	152	.563	.684	27	174	.786
195	32	.164	52	.266						169	.860
225	45	.200	63	.280						175	.781
218	42	.194	62	.284						185	.848
243	42	.172	65	.267						207	.851
192	40	.208	52	.270						163	.847
216	42	.194	65	.300						185	.856
192	42	.218	52	.270						158	.827
196	42	.214	56	.285	102	140	.520	.714	38	155	.791
246	53	.215	72	.292	111	172	.451	.699	61	206	.836
205	36	.176	52	.254	71	122	.346	.595	51	174	.847
215	42	.195	62	.288	111	147	.516	.683	36	188	.874

Len.	N.R.		Ex.P.		I.B.					A.P.	
	Dis.	%	Dis.	%	Dis.		%		Len.	Dis.	%
199μ	41μ	.206	60μ	.301						168μ	.844
216	39	.180	58	.268						168	.861
211	48	.224	66	.312						175	.829
210	40	.190	63	.300	83μ	135μ	.395	.642	53μ	169	.805
242	46	.190	67	.276						200	.831
236	46	.195	72	.305						195	.829
200	45	.225	66	.330						162	.810
209	45	.215	62	.296						174	.832
216	42	.194	60	.277						185	.856
232	51	.219	69	.297						184	.792
230	50	.217	76	.330						188	.819
245	50	.204	72	.293	126	167	.514	.681	41	205	.836
226	50	.221	75	.331						198	.877
209	48	.229	62	.296						172	.824
229	45	.196	68	.296	115	161	.502	.703	46	195	.852
221	37	.167	55	.248	86	126	.389	.570	40	185	.835
245	52	.212	80	.314	112	155	.457	.628	43	183	.829
185	43	.232	63	.340	81	125	.437	675	44	158	.856
221	42	.190	65	.294	102	154	.461	696	52	188	.849
225	46	.204	67	.297						177	.788
222	43	.192	66	.296						180	.810
270	55	.203	82	.303	145	187	.537	.692	42	214	.792
195	40	.205	59	.302						170	.869
252	46	.182	72	.285						222	.880
260	52	.200	80	.307						215	.826
278	56	.201	82	.294						233	.837

Len.	N.R.		Ex.P		I.B.					A.P.	
	Dis.	%	Dis.	%	Dis.		%		Len.	Dis	%
262µ	43µ	.164	72µ	.240						220µ	.389
230	45	.195	65	.282						188	.817
200	46	.230	65	.325						166	.831
286	70	.244	100	.349	150µ	200µ	.524	.699	50µ	233	.810
175	35	.200	52	.297	85	101	.485	.577	16	147	.840
198	35	.176	52	.262	102	135	.515	.681	33	157	.792
190	40	.210	58	.305	98	126	.515	.663	28	156	.819
251	45	.183	72	.286	117	151	.466	.601	34	200	.797
168	35	.208	50	.297	90	110	.535	.654	20	140	.832
171	32	.187	50	.292						141	.829
199	40	.201	58	.291	100	130	.502	.653	30	170	.855
165	32	.193	46	.278	79	110	.478	.666	31	138	.836
180	33	.183	52	.288	98	125	.544	.694	27	142	.789
161	32	.198	46	.285	82	109	.509	.677	27	133	.824
190	33	.173	52	.273						156	.820
169	30	.177	46	.272	86	108	.508	.639	22	136	.805
190	33	.173	51	.272	90	118	.473	.621	28	157	.826
178	35	.196	52	.292	79	101	.442	.567	22	146	.820
162	30	.185	47	.290	92	111	.566	.685	19	135	.831
182	35	.192	52	.285						152	.837
160	32	.200	49	..306						136	.852
172	32	.184	45	..261	95	113	.552	.656	18	144	.835
173	32	.184	50	.289	95	115	.549	.664	20	144	.830
196	42	.214	60	.306	112	147	.571	.750	35	160	.818
285	65	.228	95	.333						225	.790
321	65	.202	105	.330						270	.842
299	65	.217	96	.354	169	190	.565	.668	21	240	.806

Len.	N.R.		Ex.P.		I.B.					A.P.	
	Dis.	%	Dis.	%	Dis.		%		Len.	Dis.	%
295mm	56mm	.189	77mm	.261	155mm	190mm	.518	.644	35mm	243mm	.823
282	60	.212	85	.301						225	.801
290	56	.193	82	.282	145	182	.500	.627	37	253	.872
288	55	.190	82	.284						233	.809
288	53	.184	82	.284						242	.842
283	60	.212	85	.300						180	.805
222	38	.171	62	.279						184	.830
202	38	.188	60	.297						160	.793
218	36	.165	56	.257						178	.818
215	45	.209	65	.302						172	.843
200	40	.200	60	.300						167	.839
332	68	.204	100	.301						283	.823
318	70	.220	105	.330	183	215	.575	.676	32	255	.804
276	57	.206	80	.289						220	.798
328	68	.207	100	.304	155	198	.472	.602	43	272	.830
298	55	.187	93	.312	128	158	.429	.530	30	248	.834
301	58	.192	100	.332						248	.825
286	56	.195	85	.297						230	.803
295	60	.203	90	.305						247	.839
272	54	.197	80	.293						229	.842
290	56	.193	98	.337						255	.879
274	58	.211	90	.328						229	.835
291	52	.178	80	.274	152	192	.522	.659	40	236	.818
292	51	.174	86	.294	150	188	.517	.641	38	234	.803
301	66	.219	101	.335						248	.824
320	61	.190	98	.306						258	.805

FULLEBORN'S TABLES COMPARED

The average measurements for the anatomical fixed points - as given of page 27 - could then be compared with those of Fülleborn. Comparison with Fülleborn's Mikrofilarian of the Bancrofti type - group numbers, I to XI - are as follows: (His measurements come first; those from mine are second; and the difference is last.)

I. Microfilaria nocturna with pronounced periodicity (Case of St. Thome).

N.R.	Ex.P.	A.P.	Length	No. of Individuals
19.2	29.2	83.0	260.5	5
19.7	29.5	82.8	232.8	
.5+	.3+	.2-	27.7-	

II. As I.

19.1	29.4	81.6	285.7	5
19.7	29.5	82.8	232.8	
.6+	.1+	1.2+	52.9-	

III. As I

19.1	29.3	81.2	168.4	6
19.7	29.5	82.8	232.8	
.6+	.2+	1.6+	64.4+	

IV. Microfilaria with pronounced nocturnal periodicity (Case of Garin from German New-Guinea).

18.3	28.6	83.5	232.5	5
19.7	29.5	82.8	232.8	
1.4+	.9+	.7-	.3+	

V. As IV.

20.0	30.2	82.2	191.2	4
19.7	29.5	82.8	232.8	
.3-	.7-	.6+	41.6+	

VI. Microfilaria from German East Africa (Material collected by Dr. Manteufel).

N.R.	Ex.P.	A.P.	Length	No. of Individuals
19.9	30.1	82.6	263.7	28
19.7	29.5	82.8	232.8	
.2-	.6-	.2-	30.9-	

VII. Microfilaria from German East Africa (Case of Karl)

19.9	29.4	84.8	198.2	6
19.7	29.5	82.8	232.8	
.2-	.1+	2.0-	34.6+	

VIII. As VII.

19.1	28.8	81.4	187.3	6
19.7	29.5	82.8	232.8	
.6+	.7+	1.4+	45.5+	

IX. Microfilaria from the Philippines.

19.6	29.9	80.5	162.5	6
19.7	29.5	82.8	232.8	
.1+	.4-	2.3+	70.3+	

X. Microfilaria from Samoa without periodicity

19.3	28.5	82.9	272.9	5
19.7	29.5	82.8	232.8	
.4+	1.0+	.1-	40.1-	

XI. As X.

20.1	29.6	82.5	291.4	6
19.7	29.5	82.8	232.8	
.4-	.1-	.3+	58.6-	

Average.

				Sum of Individuals
19.6	29.6	82.4		82
19.7	29.5	82.8		
.1+	.1-	.4+		

Although differences here are slight, and in the average the greatest discrepancy is but .4 %, comparisons would not be complete until Fülleborn's tables for Microfilaria diurna and loa, group numbers XIII to XVIII, had been examined.

XIII. Microfilaria diurna with pronounced periodicity (Case of Peter Ahanda).

N.R.	Ex.P.	A.P.	Length	No. of Individuals
22.0	31.6	82.3	233.7	6
19.7	29.5	82.8	232.8	
2.3 −	2.1 −	.5 +	.9 −	

XIV. Microfilaria diurna (all test material from Sir Patrick Manson Inst.)

21.4	31.8	81.6	152.5	10
19.7	29.5	82.8	232.8	
1.7 −	2.3 −	1.2 +	80.3 +	

XV. Microfilaria from uteris of Loa I.

21.8	30.4	82.3	239.7	4
19.7	29.5	82.8	232.8	
2.1 −	.9 −	.5 +	6.9 −	

XVI. Microfilaria from the peripheral blood of carriers of Loa I.

21.4	31.7	82.4	143.6	5
19.7	29.5	82.8	232.8	
1.7 −	2.2 −	.4 +	89.2 +	

XVII. Microfilaria from the uteris of Loa II.

21.3	31.9	81.5	246.1	7
19.7	29.5	82.8	232.8	
1.6 −	2.4 −	1.3 +	13.3 −	

XVIII. As XVII.

N.R.	Ex.P.	A.P.	Length	No. Of Individuals
21.8	31.8	81.7	192.4	7
19.7	29.5	82.8	232.8	
2.1 −	2.3 −	1.1 +	40.4 +	

Average.

			Sum of Individuals
21.6	31.6	81.9	39
19.7	29.5	82.8	
1.9 −	2.1 −	.9 +	

Tabulation of differences obtained from the averages of both types:

	N.R.	Ex.P.	A.P.
Microfilaria of bancrofti type:	0.1 %	0.1 %	0.4 %
Microfilaria diurna and loa:	1.9	2.1	0.9

In comparing the results of the different percentages here obtained, it shows clearly that my measurements are more closely allied to the bancrofti type than to diurna and loa. But Fülleborn's blood examinations are perhaps rather few from which to draw definite conclusions, and further observations may easily modify some of his results. In the more extended of his tables, percentages are found to widely overlap, and a comparison of one hundred individuals, from which my average was made, to that of five, five, six, etc., of his groups from German New-Guinea, Samoa, the Philippines, etc, or even his rather indiscriminate average of all these, making eighty-two individuals in all, is hardly comparable or discreet. Comparisons with other types are not recorded here, for the differences with them was so marked.

Breinl (1915) in his examination of the "Occurrence and Prevalence of Diseases in British New Guinea" has made the mistake

of measuring but fourteen parasites, stiking an average, and then comparing them with Fülleborn's measurements for typical Microfilaria nocturna, group IV., obtained from but five individuals. His results are shown here:

	N.R.	Ex.P.	A.P.	Length
Breinl's average:	18.7	28.5	86.0	362.
Fülleborn's average:	18.3	28.6	83.5	232.5
	.4+	.1−	2.5+	129.5+

Next he compared his average with that of Fülleborn's non-periodic Samoan Microfilaria, group X, again but five individuals in that.

	N.R.	Ex.P.	A.P.	Length
Breinl's average:	18.7	28.5	86.0	362.
Fülleborn's average:	19.3	28.5	83.9	272.5
	.6−	.0	3.1+	89.5+

Breinl wisely does not draw any conclusive evidence from these comparisons, but relies on morphological similarities, and coincident experiences and observations with those of Bahr (1912) and Fülleborn (1911) in Fiji and in the South Pacific.

DIAGNOSTIC POINTS BETWEEN FILARIA BANCROFTI AND FILARIA LOA AS SHOWN BY DRIED AND STAINED FILMS.

Fülleborn's (1913 b) discussion of the diagnostic differences rest on the following points:

a. The periodicity. The assumption that the Microfilaria appear regularily in the peripheral blood exclusively by night, may doubtless satisfy, but other matters and conditions may enter in; even in cases having an absolutely "clear periodicity", the evidence is insufficient. Low (1909) in his work in the south seas, found that the bancrofti larva, generally speaking, possessed little periodicity. Frequently, even in abundant infection where counts were made in the blood, a single nocturnal Microfilarian will be found by day, and likewise a single diurna Microfilarian by night. Irregularity in the amnner of living, likewise the illness of carriers of nocturna and diurna will abolish the periodicity.

Notes on Illinois Case.

The smears made in the daytime, as elsewhere reported, possessed but two individuals, while if similar smears had been made at night, hundreds of embryos would have been found.

b. The bancrofti embryo is larger than loa. Fülleborn does not make as much of this point as does Low (1909).

This could not well be used as a diagnostic point in this study, for the age of the embryos will make considerable difference, also a slight shrinkage takes place in a few of the smears. The great variance in the measurements of the animals may be due in part to both of these: minimum 160 microns, and maximum 330 microns.

c. As Manson emphasized, in thick, haematoxylin-stained, dried preparations, the bancrofti embryos as a rule lie in close, spiral coils like a metal shaving; whereas loa is rumpled in wavy lines like a piece of damp woolen yarn. This point Fülleborn considers enough for diagnosis.

As the camera lucida drawings which were made for measurement showed that such a wide varity of attitudes could be assumed, it did not seem that any decision could be arrived at through this point. (See Plate.)

d. Most of the loa embryos in the thick, dried, haematoxylin preparations are more shrunken or shrivelled up than bancrofti; the central column is much wider and is not surrounded by a clear space, whereas in bancrofti the central column of nuclei is more compact and has a clear area on either side.

None of the embryos seem to be greatly shrunken; and in all cases the central column of nuclei was somewhat compact and surrounded by a clear space.

e. It follows from the point just previous, that owing to the fact that the nuclei in bancrofti are more compact than those in loa, the single nuclei can not be as readily distinguished from each other, while in loa, the nuclei are larger and fill up the entire space within the cuticula. Fülleborn cannot confirm the statement that the colorability of the nuclei of diurna is less than that of the diurna, for such a characteristic separating each can hardly be of great value because of changes with age and treatment.

In most of the emoryos, the separate nuclei, although compact, could be distinguished from each other quite plainly because of the deep staining.

f. As a rule, the central column of Microfilaria diurna seems to be broken off at the head end, whereas in nocturna, there is a gradual transition into the nuclear free part which is at the head end. Fülleborn often found in both the reverse, and an abrupt or gradual ending of the central column will perhaps be associated with the changes in the attitudes of the animal.

In none of the specimens studied did there appear to be an abrupt ending of the central column, but in all there was a rather gradual transition.

g. Fülleborn cannot confirm the theory that with loa the end of the tail is always turned in, contrary to that recorded of bancrofti.

No specimens showed the tail to be turned against the body as indicative of loa.

h. It seems to be a good discrimination between bancrofti and loa, that with bancrofti the end of the tail is free of nuclei, while in loa they reach the very end. Unfortunately in dried specimens the end of the tail of the Microfilarian is not always recognized throughout with sufficient clearness so that this characteristic loses its practical value in strongly colored preparations.

In all instances where the character of the tail was at all disernable the nuclei terminated at a point short of the tail about 5 % of the length of the body.

Fülleborn (1913 b), for practical purposes, thinks that a correct diagnosis may be made from these notations alone:

	Filaria bancrofti Embryos
1. In thick, not too slowly dried haematoxylin preparations	1.Close spiral coils

2. In Azur II preparations	2. G.1 cell relatively small

Rodenwaldt (1909) presents some diagnostic points of value in connection with the position of the excretory and genital cells and the relation of them to their respective excretory and anal pores:

The excretory cell of the Microfilaria diurna is spindle shaped, long drawn out, with narrow, long, oval shaped nucleus, sending a strand to the excretory pore which lies at least two widths of the worm from it. Entirely the opposite with the Microfilaria nocturna. Its excretory cell is nearly mediate to the excretory pore, and appears to lie just outside the opening vacuole. The nucleus of the cell lies accordingly scarsely a worm's breadth behind the pore. It is broad, oval, plump, and the plasma surrounds it in a compact mass so that the real spindle form is rarely evident and the form of the whole cell is rather more rectangular.

> In the specimens examined, where an excretory cell was evident, its proximity to the pore was very marked, for it was found in all cases to be lying immediately beside the vacuole. The shape was broadly oval, at no time approaching the long drawn-out spindle shape of the loa.

The chief genital cell, G.1, of loa is recognizable by its large size, while in bancrofti it is much smaller and easily overlooked. The other genital cells, G.2,3,4, in loa are some little distance from the anal pore; while in bancrofti, G.4 is mediate to the pore, and the others extend anteriorly in close serial form, with G.1 some little distance from G.2.

> As described previously in the morphology of the Microfilaria under examination, the genital cells and their relation to the anal pore, follow Rodenwald's description of

the conditions diagnostic of bancrofti.

With giemsa staining, the sheath takes on a pale violet tint; the central body appears as a continuous granular filament, staining a bright violet-red. The central column of cells, stained a violet-blue, is narrow, and so leaves a clear space on either side of it. The tail appears drawn out, is sharply pointed, and the terminal cells in most cases fall short of the end of the body by a distinct interval. (See Plate.)

From Foley's tabular form, (1913) the diagnostic characteristics for the embryos of Filaria bancrofti correspond with the conditions which I found to exist.

Stained by giemsa method	Filaria bancrofti embryos
Sheath	Colored
Cells	Violet-blue
Cells of cephalic extremity	Sharply terminated in a straigh row
Cells of the body	Small, rounded, easy to count forming a column narrower than body of embryo
Cells of caudal extremity	Not reaching the point of tail
Central viscus	Well colored

Summary of features showing diagnostic evidence of Microfilaria bancrofti.

1. Marked periodicity.

2. Central column of nuclei somewhat compact and surrounded by a clear space.

3. End of tail free of nuclei.

4. Excretory cell almost rectangular in shape, lying immed-

iately posterior to and touching the pore.

5. G.1 cell relatively small.

6. G.2,3,4, cells small and arranged in serial form directly anterior to anal pore.

7. Inner body well colored with giemsa staining.

CONCLUSIONS.

The real problem of this study is the identification of the organism involved in an Illinois case of filariasis in a native of British Guiana.

As previously shown, the question lies between Microfilaria bancrofti and Microfilaria loa; no one characteristic can determine a valid differential diagnosis between these, but a close agreement in measurements with Fülleborn's tables of the bancrofti type are indicatory of that organism.

The correspondence in morphological details as depicted by authoritative investigators as Castellani and Chalmers (1913), Fantham, Stevens, and Theobald (1916), Fülleborn (1911, 1913 a and b), Looss (1914), Rodenwaldt (1908 and 1909), and Wood (1905) serve to show very conclusively, along with the pathological significance and periodicity, that the embryos can only be those of Filaria bancrofti.

EXPLANATION OF PLATE

Characteristic attitudes of Microfilaria, drawn with camera lucida.

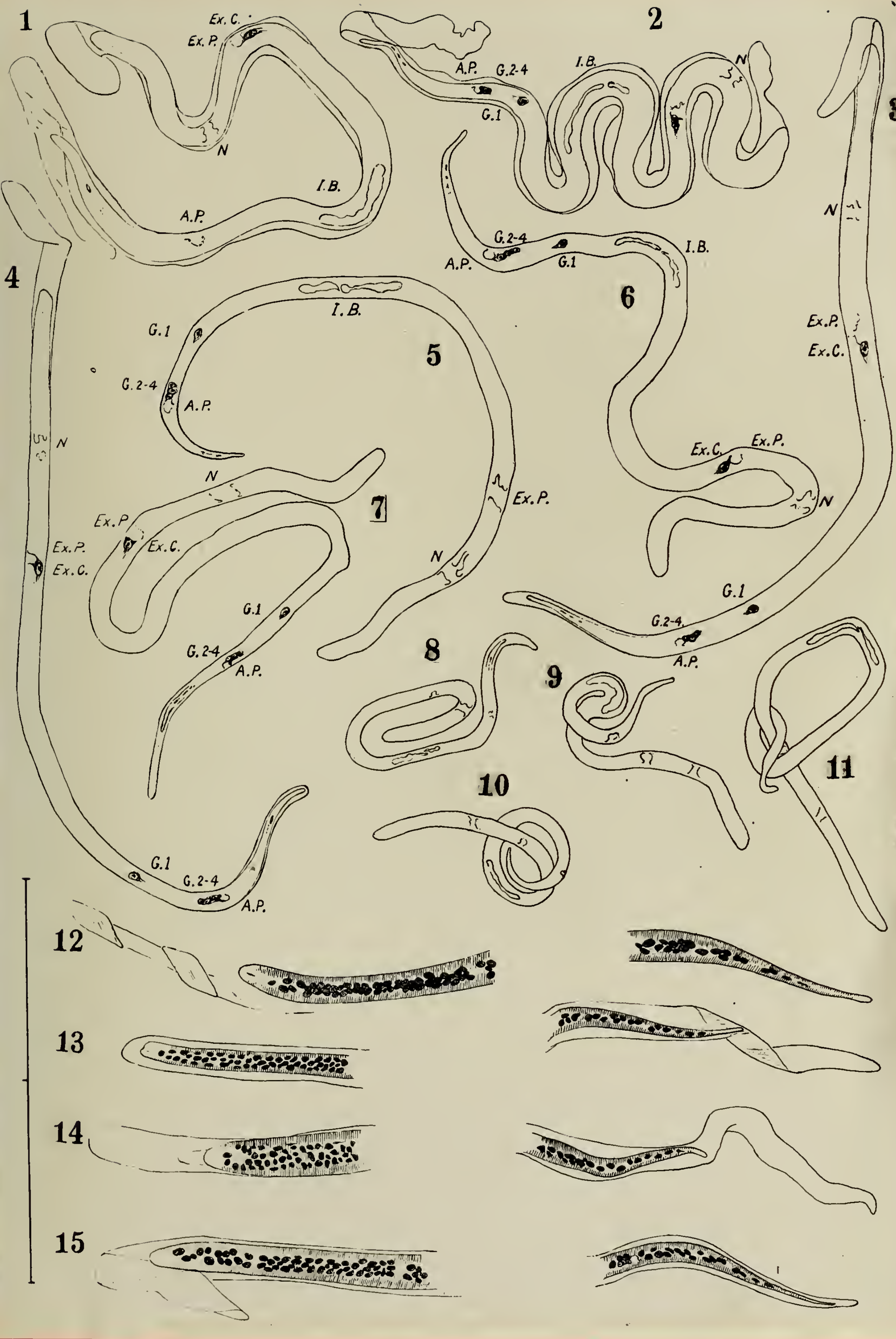
Figures 1 - 7, Ocular 4, Oil immersion 1/12

Figures 8 - 11, Ocular 4, Objective 8

a p	anal pore
ex c	excretory cell
ex p	excretory pore
g 1	chief genital cell
g 2,3,4	genital cells:2,3,4
i b	inner body
n	nerve ring

Figures 12 - 15, Ocular 4, Oil immersion 1/12

Heads and Tails, showing positon of nuclei.


1
Ex. C.
Ex. P.
N
I.B.
A.P.
2
A.P.
G.2-4
G.1
I.B.
N
3
N
Ex.P.
Ex.C.
G.1
G.2-4
A.P.
4
N
Ex.P.
Ex.C.
G.1
G.2-4
A.P.
5
I.B.
G.1
G.2-4
A.P.
Ex.P.
N
6
G.2-4
A.P.
G.1
I.B.
Ex.C.
Ex.P.
N
7
N
Ex.P
Ex.C.
G.1
G.2-4
A.P.
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